A STUDY OF THE PREVALENCE OF CAMPYLOBACTER PYLORI IN PATIENTS WITH UPPER GASTROINTESTINAL SYMPTOMS, AND AN EVALUATION OF VARIOUS LABORATORY METHODS TO DETECT ITS PRESENCE.

A project submitted to the Department of Medical Microbiology, Faculty of Medicine, University of Natal, Durban in partial fulfilment of the requirements for the degree of Master of Medicine (Microbiology).

Durban 1988
I declare that this project is my own work.

This project has not been submitted in part or in whole for any degree or examination to any other university.

The studies described in this project were carried out at the Faculty of Medicine, University of Natal, Durban, under the supervision of Professor Jan van den Ende.

N M MILLER

DATE

13 OCTOBER 1988
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ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to the following individuals for their assistance in the preparation of this project:

Professor J van den Ende, supervisor, Head of the Department of Medical Microbiology, University of Natal, for his expert guidance, encouragement and constructive criticism.

Professor A E Simjee and members of the Gastrointestinal Unit, Department of Medicine, without whose assistance these studies would not have been possible.

Dr A Naran, Department of Anatomical Pathology, University of Natal, for examining the histological preparations.

M A Sathar, Gastrointestinal Unit, University of Natal, for performing the serological assays.
Antral mucosal biopsies were examined microbiologically and histologically for the presence of Campylobacter pylori in 224 patients with upper gastrointestinal symptoms. One hundred and eighty three (83%) patients were found to harbour Campylobacter pylori in their gastric mucosa. Campylobacter pylori was strongly associated with the presence of histological gastritis (93%) and was detected in only 10% of 30 patients whose gastric biopsies showed normal histology. Endoscopically diagnosed duodenal lesions were more strongly associated with the presence of Campylobacter pylori than were gastric lesions (p <0.001).

A variety of laboratory methods were evaluated to determine the sensitivity and specificity to detect the presence of Campylobacter pylori. Histology was the most sensitive and specific method to detect the presence of Campylobacter pylori. Although culture was highly specific, it was less sensitive than histology in detecting Campylobacter pylori in gastric antral mucosal specimens. The "conventional" gastric urease assay, although specific, needs be performed under controlled conditions (37°C) for optimal results. The "one-minute" urease assay was more sensitive than the "conventional" gastric urease assays and was highly specific. ELISA to detect specific-IgG antibodies to Campylobacter pylori was a moderately sensitive non-invasive
method to detect *Campylobacter pylori* infection, but was non-specific.
INTRODUCTION

Although the presence of spiral bacteria on gastric mucosa of post-mortem specimens was noted by histopathologists in the early part of this century (1), their possible role as pathogens was never clarified. It was only after Warren and Marshall (2) reported the isolation of a Campylobacter-like microorganism, now named Campylobacter pylori (C. pylori) (3), from the gastric mucosa of humans in 1983, that worldwide interest and research into their possible role in peptic ulcer disease and gastritis was initiated.

The strong association between these bacteria and histological gastritis and duodenal ulceration has since been confirmed by many investigators worldwide (2,4 - 10). Little, however, is known about its occurrence in South African patients.

There is now strong evidence that C. pylori causes gastritis (11,12) and that treatment with antimicrobial agents such as bismuth salts in combination with ampicillin, metronidazole, tetracycline, erythromycin or furazolidine may be followed by eradication of this organism, normalisation of gastric histology and may result in lower relapse rates of peptic ulceration when compared to the use of H2-antagonists alone (13 - 17).

Diagnosis of C. pylori infection has largely been based on
isolation of these bacteria from gastric biopsy specimens obtained at endoscopy or by direct visualisation on stained tissue sections. The results of both culture and histology may only be available after several days and thus efforts have been made to establish more rapid techniques and to develop non-endoscopic methods to detect the presence of C. pylori, so as not to delay the early initiation of specific therapy.

A prominent feature of C. pylori is the production of large quantities of extracellular urease (5,18). This enzyme has been postulated to play an important role in the pathogenesis of gastritis and peptic ulceration (19,20). Investigators have also used this property of C. pylori as an indirect, rapid marker of the presence of C. pylori in biopsy specimens (21 - 24).

Since persons infected with C. pylori develop both local and serum antibodies to this organism (25 - 28), much interest has been devoted to serological tests to detect antibody to C. pylori as a potential non-endoscopic approach to diagnose C. pylori infection.

Despite growing interest in C. pylori-associated gastrointestinal disease, optimal methods to detect its presence have not been established.

This paper reports the findings of three separate studies: the first, a prospective study, was designed to determine the
prevalence of \textit{C. pylori} in a series of patients with upper gastrointestinal symptoms, referred for endoscopy, at King Edward VIII Hospital in Durban; the second and third parts of the study were designed to investigate the sensitivity and specificity of various techniques to detect the presence of \textit{C. pylori}. 
PATIENTS AND METHODS

Two hundred and twenty four newly presenting African and Indian patients with upper gastrointestinal symptoms for which endoscopy of the upper gastrointestinal tract was indicated, were included in the first part of the study. Informed consent was obtained from all patients and the study was approved by the Ethics Committee of the Faculty of Medicine, University of Natal. During endoscopy, two antral mucosal biopsies were taken from within 5 centimeters of the pylorus. One was placed into brucella broth for bacteriological culture and the other into 10% formalin prior to histological examination. The endoscopic diagnoses were recorded and known only to the endoscopist until the end of the study.

Histology: Formalin fixed biopsies were routinely processed and sections were stained with both haematoxylin and eosin (H & E) and by the Warthin-Starry technique and examined for both the presence of gastritis and "campylobacter-like organisms". On histological examination, any gastritis detected was classified into chronic superficial and chronic atrophic gastritis. The pattern of inflammation was further categorised as "active" or "inactive". "Active" gastritis required the presence of polymorphs, plus lymphocytes and plasma cells infiltrating the lamina propria together with degenerative changes; when
polymorphs were absent, the gastritis was classified as "inactive". *C. pylori* were usually recognised as curved or spiral organisms, usually lying in the mucous layer and adjacent to the epithelium, especially in the gastric glands and at the junctions between the cells (plates 1 and 2).

Microbiology: Antral biopsies in brucella broth were finely minced with sterile blades and inoculated onto chocolate agar within 3 hours of collection. The plates were then incubated microaerophilically, using "Gaspak" envelopes without catalyst (BBL Microbiology Systems, Cockeysville, MD), for up to 10 days and were examined on days 3, 7 and 10 for growth. Tiny clear colonies (plate 3) were identified as *C. pylori* by Gram stain, positive oxidase test, positive catalase test and characteristic rapid urease reaction (5). A specimen was considered to be negative for *C. pylori* if no growth occurred within 10 days incubation. The initial 40 specimens were also cultured on a selective medium, Skirrows formulation of campylobacter agar (29). Initial isolates were also tested for sensitivity to nalidixic acid and cephalothin.

Endoscopes and biopsy forceps were disinfected with 2% gluteraldehyde solution and rinsed with sterile water between examinations. Bacteriological testing of the instruments after decontamination yielded no growth of *C. pylori*, indicating that this bacterium was not being transferred between patients.
In the second study, microbiological culture, histology and gastric urease assays, which required the taking of gastric antral biopsy specimens at the time of upper gastrointestinal endoscopy, and an enzyme-linked immunosorbent assay for the detection of specific-IgG antibodies to *C. pylori* in serum specimens were evaluated. One hundred newly presenting patients, in whom upper gastrointestinal endoscopy was conducted for symptoms referrable to the upper gastrointestinal tract, were included in the study. At endoscopy, three antral mucosal biopsies were taken from each patient within 5 centimeters of the pylorus. These were processed for histology, microbiological culture and gastric urease assays. A sample of venous blood was collected from each patient for measurement of antibodies to *C. pylori*.

Specimens for histological examination were processed as in the first part of the study. However, sections were stained only with H & E. The Warthin-Starry technique was discontinued in this part of the study.

**Microbiological culture and gastric urease assay at 37°C:**

A second antral biopsy was transported in brucella broth to the microbiology laboratory and usually processed within 3 hours of collection. The specimen was first inoculated onto chocolate agar and then placed into 2 ml of 2% Christensen's urea broth (30). Inoculated chocolate agar plates were incubated as in the first
part of the study and suspect colonies identified by positive oxidase, positive catalase and rapid urease reactions (5). Inoculated Christensen's urea broth was incubated at 37°C overnight to detect preformed urease activity. The principle of the gastric urease assay is that preformed urease in the biopsy specimen, colonised or infected with \textit{C. pylori}, converts urea to ammonia. This, in turn, leads to a pH change in the test medium and to a colour change of the phenol red indicator. A colour change from yellow to pink or orange indicated a positive test. The specimen was considered to be urease negative if no colour change developed after 18 hours incubation.

Gastric urease assay at room temperature:

A third gastric antral biopsy specimen was placed immediately, in the endoscopy room, into 2 ml of 2% Christensen's urea broth and kept at room temperature for up to 18 hours before being examined for any colour change, as described above.

Serology:

Antigen preparation: Stored strains of \textit{C. pylori} were grown on chocolate agar for 5 days in a microaerophilic environment, harvested in normal saline, centrifuged at 1500 x g for 10 minutes, washed twice in phosphate buffered saline (PBS pH 7.2) and then fixed in 4% formaldehyde, washed again and stored at 4°C in PBS with 0.1% sodium azide for a maximum of 2 days until used as antigen.
ELISA: The indirect enzyme-linked immunosorbent assay (ELISA) was used to measure anti-*C. pylori* IgG antibodies (31). Each well of polystyrene flatbottom microtitre plates (Dynatech M129, T & C Scientific Supplies, Durban) were coated with 100 μl of 18.75 x 10⁶ formalized intact cells of *C. pylori* in coating buffer (0.05M carbonate pH 9.6) and incubated for 2 hours at room temperature in a humid container. Plates were washed with 0.05M tris-0.1M NaCl buffer (TS)(pH 8.0) containing 0.05 % (w/v) Tween 20 (TST) in an ELISA shower (32). Excess TST was "flicked off" and the plate was padded dry on absorbent material.

Fifty microlitres of patient's serum, diluted 1/100 in TS, was then added to duplicate wells and incubated for 1 hour at 45°C in a humid container. The plates were washed with 500 ml of TST, dried and incubated with 50 μl of a 1/1000 dilution of gamma-chain specific goat anti-human IgG conjugated to horse radish peroxidase (Sigma Chemicals, St Louis, USA) for 1 hour at 45°C in a humid container. The plates were washed as previously described and 50 μl of chromogenic substrate (hydrogen peroxide/ortho-phenylene diamine) (33) was added to each well and plates were incubated for 5 minutes in the dark at room temperature. The reaction was stopped by adding 50 μl of 1.5N hydrochloric acid (HCl) (33) to each well. The optical density (OD 492 nm) was measured using a Titertek Multiscan MicroELISA plate reader (Flow Laboratories). Absorbance values of 0.300 or more were regarded as positive, whereas values of less than 0.300 were
regarded as negative.

In the third part of the study, histology, microbiological culture, the gastric urease assay performed at 37°C, and a "one-minute" gastric urease assay (24) were evaluated in 100 consecutive patients with upper gastro-intestinal symptoms referred for upper gastrointestinal endoscopy. Gastric antral mucosal biopsy specimens for histology, microbiological culture and gastric urease assay at 37°C were performed as in the first and second part of the study. In addition, a third gastric antral biopsy specimen was placed immediately, in the endoscopy room, into a Bijoux bottle containing 1 ml of 10% w/v urea in deionised water at pH 6.8, and 2 drops of 1% phenol red. A positive result was recorded if the colour changed from yellow to pink within one minute.
RESULTS

C. pylori isolates, throughout the study period, fulfilled the criteria reported by others (3,6,7), being Gram-negative, curved or U-shaped, oxidase positive, catalase positive and a strongly urease positive. All initial isolates were resistant to nalidixic acid and sensitive to cephalothin on disc diffusion testing.

First part of study

Of the 224 patients studied in the first part of the study, C. pylori was cultured in 146 (65%) and seen on histology in 179 (80%) of the patients. In 183 (82%) the microorganism was either cultured and/or seen on histology. In only 4 patients was the microorganism cultured but not seen on histology.

The association of C. pylori with endoscopic findings is shown in table I. C. pylori was cultured and/or seen on histology in 65 (97%) of 67 patients with duodenal ulceration, 19 (95%) of 20 patients with duodenal erosions, 9 (90%) of 10 patients with duodenal erythema, 13 (62%) of 21 patients with gastric ulceration, 1 (33%) of 3 patients with gastric erosions and in 8 (62%) of 13 patients with gastric erythema. C. pylori was detected in 53 (76%) of 70 patients in whom endoscopy was reported as normal and fifty (71%) of these 70 patients had
histological evidence of gastritis.

The association between the presence of \textit{C. pylori}, as detected by culture and / or histology, and histologically diagnosed gastritis is shown in table II. \textit{C. pylori} was detected in 167 (94\%) of 177 patients with chronic superficial, active gastritis, in 10 (83\%) of 12 patients with chronic superficial gastritis without activity and in 3 (60\%) of 5 patients with chronic superficial gastritis with intestinal metaplasia. Thus, \textit{C. pylori} was detected in 180 (93\%) of 194 patients with histological evidence of gastritis. In contrast, the microorganism was cultured and / or seen on histology in only 3 (10\%) of 30 patients with normal antral histological features. In 1 of the 3 patients, the microorganism was both cultured and seen on histology; in the other 2 patients it was cultured but not seen on histology. The latter may reflect a sampling error due to the patchy distribution of gastritis or result from low numbers of organisms in the biopsy specimen sent for histology.

One hundred and thirty of 152 male patients (86\%) were \textit{C. pylori}-positive in contrast to 53 (74\%) of 72 females studied. This difference was significant ($p < 0.05$) (table III). There was no statistically significant difference in the prevalence of \textit{C. pylori} in Indian and African patients ($p = 0.38$) (table IV).

Second part of study

\textit{C. pylori} was cultured in 57 and seen on histology in 82 of
the 100 patients studied in the second part of the study. In 84 patients _C. pylori_ was either cultured and / or seen on histology. _C. pylori_ was cultured but not seen on histology in only 2 patients.

Detection of _C. pylori_ by culture and / or histology, or both, was taken as the "gold standard" with which gastric urease assays and serology, to detect specific-IgG antibody to _C. pylori_, were compared.

Table V shows that histology was the most sensitive and specific technique evaluated to detect _C. pylori_ infection. Although culture was 100% specific, it was much less sensitive than histology (68% vs 98%). The gastric urease assay performed under controlled conditions (37°C) was more sensitive (71% vs 55%), but less specific (81% vs 100%) than when performed at room temperature. Although detection of specific-IgG antibodies to _C. pylori_ in sera was relatively sensitive (82%), this test had low specificity (38%). Specific-IgG antibodies to _C. pylori_ were detected in 10 (62.5%) patients in whom the organism was neither detected by culture nor histology.

The correlation between histologically diagnosed gastritis and the detection of _C. pylori_ by the various methods evaluated is shown in table VI. Chronic, superficial active gastritis was detected in 90 patients. In 10 patients, no abnormality was detected on histological examination. Chronic, superficial
inactive gastritis and chronic atrophic gastritis were not detected in any of the patients studied. *C. pylori* was detected in 80 (89%) of patients with histological evidence of gastritis by histology and/or culture. In contrast, this microorganism was cultured or seen on histology in only 2 (20%) of patients with normal histology. The gastric urease assays detected *C. pylori* in 64% and 51% of patients with histological gastritis when performed at 37°C and room temperature respectively. Specific-IgG antibodies to *C. pylori* were detected in 76% of patient with histological gastritis; serology also appeared relatively specific (90%) in the detection of *C. pylori*-associated gastritis. A positive antibody result was uncommon (10%) in the absence of histological gastritis.

Third part of study

One hundred newly presenting patients were included in the third part of the study (table VII). *C. pylori* was detected in 88 by culture and/or histology. Histology again was the most sensitive technique evaluated to detect the presence of *C. pylori*. Culture detected 79% of *C. pylori*-positive patients. The "one-minute" gastric urease assay detected 86% of *C. pylori*-positive patients and was 100% specific in contrast to the gastric urease assay performed at 37°C, which detected 70% of *C. pylori*-positive patients.
DISCUSSION

C. pylori is a redescribed microorganism which has been implicated in gastroduodenal disease. It is a microaerophilic spiral Gram-negative bacterium which is unique in producing large amounts of extracellular catalase and abundant amounts of urease (2,6,10,34). Both these factors may be important in allowing the microorganism to reside in intimate contact with the gastric mucosal cells beneath the mucous layer.

These bacteria were initially referred to as "gastric campylobacter-like organisms" (GCHO); later the scientific name Campylobacter pyloridis was proposed, since Marshall and other investigators had initially cultured it from the pyloric area of the stomach (10). This was changed to C. pylori in 1987 (3). Since the ultrastructure, flagellae and cellular fatty acid profile of C. pylori is significantly different from that of the intestinal campylobacters, further taxonomic changes are likely (18,35,36).

In this study, C. pylori was detected in 93% of patients with histological evidence of gastritis compared to 10% of patients with normal histology (table II). This is in keeping with the findings of others (2,4 - 8) suggesting that C. pylori is strongly associated with histological gastritis and is only rarely found in gastric biopsy specimens which show no evidence
of inflammation. The chronic gastritis with which \textit{C. pylori} is associated, usually involves the antrum and is commonly called non-autoimmune (type B) gastritis (37) in order to distinguish it from the gastritis found in pernicious anaemia (type A gastritis). However, there is considerable disagreement as to whether the activity of the gastritis is consistently associated with the presence of this organism. Marshall and Warren's claim (38) for a special relationship with the activity of the gastritis, as signified by the degree of polymorphonuclear leucocyte infiltration, is not supported by all investigators (6,7,39). In this study, \textit{C. pylori} was detected in 94% of patients with chronic superficial gastritis with activity, compared to 83% of patients with chronic superficial gastritis without activity (p = 0.5), suggesting that activity is not an important determinant for its presence.

\textit{C. pylori} was detected in 97% of patients with duodenal ulceration, 95% of patients with duodenal erosions, 90% of patients with duodenal erythema, 62% of patients with gastric ulceration, 33% of patients with gastric erosions and 62% of patients with gastric erythema (table I). This suggests that there is a higher correlation between \textit{C. pylori} and endoscopic observation of duodenal lesions, than between \textit{C. pylori} and endoscopic findings of gastric lesions (p <0.001). This is in agreement with the findings of several other investigators who have failed to show as strong a correlation with gastric ulcers
as there is with duodenal ulcers (5,7,8,10,38). Price et al (7) also found a higher correlation between the presence of C. pylori, in antral biopsies, with duodenal ulceration than with gastric ulceration (81% vs 57%) while Marshall et al (10) found C. pylori to be associated with 90% of duodenal ulcers and 70% of gastric ulcers. However, unlike other reports (7), C. pylori was less commonly found in patients with gastric ulcerations and gastric erythema than in patients with normal endoscopic findings (62% vs 76%).

The prevalence of C. pylori in males is significantly more common than in females (p<0.05) (table III). This confirms the findings of Rokkas et al (40). Rouvraj (41) and Rotter (University of Vienna; personal communication), however, did not show this sexual difference. There was no statistically significant difference between the presence of C. pylori in Africans and Indians (p = 0.38) (table IV).

The significance of these findings is uncertain. The statistically significant correlation between C. pylori and histological gastritis, duodenal ulceration, duodenal erythema and duodenal erosions, however, does not prove a causal relationship. Evidence for a causal relationship (i.e. pathogenicity) is provided by several recent publications. Marshall fulfilled Koch's postulates when he developed gastritis within 7 days of ingesting a culture suspension of C. pylori
isolated from a patient (11). Subsequently Baskerville and Newell were able to infect rhesus monkeys with a human strain of \textit{C. pylori} (42). Treatment studies have shown that agents with in-vitro activity against \textit{C. pylori}, such as bismuth salts, metronidazole and erythromycin are as successful as H-2 antagonists in healing peptic ulcers but with a significantly lower relapse rate (11,23,24). Drumm et al indicated that \textit{C. pylori} is specifically associated with primary antral gastritis in children and does not appear to colonise gastric mucosa secondary to inflammation such as may follow ingestion of prednisone and non-steroidal anti-inflammatory agents or accompany Crohn's disease and eosinophilic gastritis (43). Finally, IgG and IgA antibodies to \textit{C. pylori} have been shown to be present in the serum of patients harbouring the microorganism (6) and patients with duodenal ulceration have been found to have significantly higher antibody levels than healthy adults and children (25). Although these reports are only circumstantial, they seem to support the view that \textit{C. pylori} may indeed be a cause of gastritis and gastroduodenal disease. It is important, however, to develop an animal model to investigate the mechanisms underlying the \textit{C. pylori}-associated gastroduodenal disease and study the treatment thereof, without the need for human volunteers.

Many studies are in progress to assess the effects of anti-ulcer therapy and antibiotics on \textit{C. pylori}-associated gastritis
in an effort to elucidate any causal relationship with peptic ulcer disease. Reliable methods for the detection of *C. pylori* are essential to such studies.

The association between *C. pylori* and histological gastritis:

The second part of the study confirms the observations of the first part of the study, namely that *C. pylori* is strongly associated with histological gastritis, especially chronic, superficial active gastritis (table VI). It was detected by histology, culture and serology in 89%, 61% and 76% of patients with chronic, superficial gastritis respectively. These bacteria are occasionally found in fundal gastric mucosa mainly in association with chronic antral gastritis (44). In the duodenum, *C. pylori* is found only in the presence of active duodenal inflammation in areas of gastric metaplasia (45,46). Thus the association between *C. pylori* and type B gastritis seems unique, tending to support the view that this bacterium has a pathogenic role in this lesion.

Histology:

Histology was the most sensitive and specific technique studied to detect the presence of *C. pylori* infection. It was detected on histology in 98% and 97% of *C. pylori*-positive patients in the second and third parts of the study respectively (tables V and VII). The characteristic curved morphology of
C. _pylori_ and its close association with the mucosal surface allows presumptive diagnosis of _C. pylori_ in gastric biopsy specimens. These bacteria are faintly visible on H & E sections and appear to be more easily recognised in sections stained by the expensive and time consuming Warthin-Starry technique. We, however, did not use the Warthin-Starry stain (2) to detect the presence of _C. pylori_ in gastric biopsy specimens in the second and third parts of the study because this stain did not detect _C. pylori_ in sections found to be negative on H & E staining in the first part of the study. Controversy seems to exist as to which histological stain is best for the detection of _C. pylori_ in gastric biopsy specimens. McMullen et al (47) have suggested the use of the Gimenez stain in view of the poor results they obtained with the H & E and Warthin-Starry stains. Others have suggested the use of acridine orange and the Dieterle stain (9,48). The H & E stain, however, in the hands of an experienced histopathologist, appears to be adequate. Microscopic techniques, which have been employed by others, but were not evaluated in these studies, include phase contrast microscopy (49), the Gram stain and indirect immunofluorescence (50).

Although histology is both very sensitive and specific, the patient cannot be treated immediately with "appropriate" drugs since it usually takes several days before a report is available.

Culture:

In all 3 studies culture was less sensitive than histology
for the detection of *C. pylori* in gastric biopsy specimens which is in accordance with the findings of others (7,47). Culture was comparable in sensitivity, but more specific than the gastric urease assay performed at 37°C and serology.

Chocolate agar, the original medium used to recover the organism (2), was used successfully in our laboratory in most instances. Although yeasts, alpha-haemolytic streptococci and enterobacteriaceae were sometimes encountered, the isolation of *C. pylori*, on non-selective chocolate agar, did not pose a problem since it was the sole organism isolated in the majority of cases. The need for a selective agar such as the Skirrow formulation of campylobacter medium, which contains 7% lysed horse blood together with vancomycin, nalidixic acid and trimethoprim has been recommended by some (51,52). This selective medium, however, did not increase the yield of *C. pylori* isolates in the initial 40 specimens cultured in the first part of the study (data not shown) and was thus discontinued. Although contamination of biopsy specimens with oropharyngeal bacteria was not a major problem, care needs to be exercised in obtaining specimens.

However, the detection of *C. pylori* by culture has obvious limitations: it is less sensitive than histology; it may take as long as 10 days for a result; and the patient must make a return visit in order to obtain treatment on the basis of culture
results - this is also applicable to histology. The lower sensitivity of culture when compared to histology in this study may be explained by the fact that the success rate of culture declines significantly if the specimen is not directly inoculated onto the growth medium (52). In addition, Goodwin et al (52) have shown that fine grinding of specimens increased the sensitivity of the culture results when compared to merely mincing the tissue. False negative findings may also have resulted from patient having taken bismuth preparations or antimicrobial agents prior to endoscopy, or may be due to patchy distribution of C. pylori in the stomach (52). In addition, culture may not permit the detection of C. pylori when few organisms are present. Although culture in our hands was less sensitive than histology for the detection of C. pylori in gastric biopsy specimens, it is the only technique which allows the accurate identification of the bacterium and makes possible the determination of susceptibility to antimicrobial agents, which is essential to the performance of therapeutic trials.

Gastric urease assays:

The urease activity of C. pylori has attracted much interest including an approach towards a rapid diagnosis (21 - 24). This urease is a high molecular weight enzyme which exhibits a high affinity for urea and has significantly higher activity than the urease produced by enteric Gram negative bacilli such as Proteus spp. and Klebsiella spp. (53).
The sensitivity of the gastric urease assay performed at room temperature (55%) is lower than that obtained by McNulty and Wise (21). This lower sensitivity may be explained by the fact that gastric antral biopsy specimens were not crushed during inoculation, but were used "whole" as obtained.

The optimum temperature of 43°C for *C. pylori* urease activity (53) suggests that more rapid detection of the organism in gastric biopsies assayed in urea broth could be achieved if tubes were incubated at 43°C. *C. pylori* urease is also more active at 37°C than at room temperature, which may explain the higher sensitivity of the urease assay obtained at 37°C (71%) in comparison to the assay performed at room temperature (55%) (table V). It would thus appear that conventional gastric urease assays should be performed at temperatures of 37°C or 43°C for optimum results.

Although the gastric urease assay performed at 37°C was not significantly more sensitive than culture (p = 0.76), this test may have been more sensitive if large numbers of organisms probably were not removed from the surface of the specimen during initial inoculation of solid (chocolate) medium prior to inoculation of urea broth, and if there had been less delay in transport to the laboratory.

Since the vast majority of positive gastric urease assays took longer than 6 hours to react positively (data not shown),
the conventional gastric urease assays (21 - 24) cannot be recommended as a reliable and rapid test to detect the presence of \textit{C. pylori} in gastric biopsy specimens. The "one-minute" urease assay was not significantly more sensitive than culture (p = 0.32) (table VII). This assay may be a potentially useful test to detect \textit{C. pylori} since, if the test if positive, appropriate therapy may be initiated immediately. There were no false positive results with the "one-minute" urease assay, which appears to be a further advantage, since other urease-producing bacteria require prolonged culture and may be unable to effect a colour change in this test. A gastric urease assay is now commercially available as the CLO test (Delta West, Western Australia). This test, however, is expensive and requires 24 hours incubation for optimal results (24).

Serology:

A serodiagnostic assay has been proposed as a primary screening procedure to detect antibodies to \textit{C. pylori} and thereby eliminating the need for endoscopy to collect gastric biopsy specimens. Methods of measuring antibodies to \textit{C. pylori} have included complement fixation tests (5), enzyme-linked immunosorbent assays (6,25 - 28,54,55) and immunoblotting (25,55,56).

Although ELISA is a well established method, the use of different antigens, such as whole bacteria, sonicated or acid-
extracted antigens have resulted in varying sensitivities and specificities.

We used whole, formalin-killed organisms (26) to detect specific-IgG to *C. pylori*. Specific-IgM was not sought because other authors have not found antibodies of this class to be useful in the serodiagnosis of *C. pylori* infection (26,54).

Specific-IgG antibodies to *C. pylori* were detected in 76% of patients with *C. pylori*-associated gastritis and results showed a specificity of 90% (table VI) with a positive predictive value of 98% and a negative predictive value of 29%. Thus a positive ELISA reaction provides strong presumptive evidence of histological gastritis. This observation is consistent with the findings of others (26,54,57). A negative ELISA, however, should be treated with circumspection, due to the low negative predictive value. As endoscopic findings alone appear to be poor indicators of *C. pylori*-associated gastritis, availability of a more specific ELISA may provide a useful screening test for the detection of *C. pylori*-associated gastritis.

In this study, 22 (24%) patients were seronegative but had *C. pylori*-associated gastritis (table VI). These false negative results are possibly due to lack of sensitivity of the ELISA used in the study.

The ELISA described was able to detect specific-IgG
antibodies to \textit{C. pylori} in 82\% of patients in whom \textit{C. pylori} was detected in gastric biopsy specimens by culture and/or histology (table V). In contrast to Dwyer et al (28), who did not detect antibodies to \textit{C. pylori} in any culture-negative patients, specific-IgG antibody to \textit{C. pylori} was also detected in 10 of 16 (62\%) patients in whom \textit{C. pylori} was not detected by culture or histology. The significance of this finding remains unclear. A possible explanation is that specific-IgG antibodies to \textit{C. pylori} may persist after elimination or clearance of the organism and resolution of the gastritis. Jones et al (27) have shown that antibody to \textit{C. pylori} may persist in some individuals for many years.

Serological cross-reactions between \textit{C. pylori} and other \textit{Campylobacter} species have been described (55), but do not appear to be relevant in the serodiagnosis of \textit{C. pylori} infection with the serum dilutions generally used (6,27,37,54,56). However, cross-reactions with other bacteria cannot be excluded as a possible explanation for the poor specificity obtained by ELISA, since absorption studies were not performed.

Before serology can be used as the "sole" test to detect \textit{C. pylori} infection, both specificity and sensitivity need to be improved. Current work in progress is designed to improve sensitivity and specificity of serological assays using monoclonal antibodies to purify specific antigens to \textit{C. pylori}. 
The approach of using \textit{C. pylori} specific urease as antigen (58) is an interesting serological method to detect \textit{C. pylori}. 
CONCLUSIONS

In conclusion, these studies confirm that *C. pylori* is strongly associated with histological gastritis and duodenal lesions. However, further investigations are needed in order to resolve whether *C. pylori* actually causes gastritis and peptic ulceration, or whether prior tissue damage enables the microorganism to act as an opportunist. Although information about *C. pylori* has accumulated rapidly, more therapeutic studies are required to determine the optimal therapies for *C. pylori*-associated gastritis and peptic ulcer disease and to fully understand its role, if any, in the aetiology of peptic ulcers. An aetiological role for *C. pylori* in peptic ulcer disease would have tremendous clinical, therapeutic and financial implications, since maintenance therapy with H2-antagonists to prevent peptic ulcer relapses is costly and imposes great demands on patients' compliance. Furthermore, these agents have no in-vitro or in-vivo activity against *C. pylori* (59). To answer the question of its role in peptic ulcer disease, highly sensitive and specific diagnostic techniques to detect *C. pylori* infection, and cooperation between gastroenterologists, microbiologists and histopathologists is needed. Since no single technique is ideal, it is strongly recommended that a combination of tests, especially those performed on biopsy specimens, should be utilised.
### TABLE I

THE ASSOCIATION BETWEEN THE ENDOSCOPIC DIAGNOSIS AND CAMPYLOBACTER PYLORI (FIRST STUDY)

<table>
<thead>
<tr>
<th>ENDOSCOPIC DIAGNOSIS</th>
<th>NUMBER OF PATIENTS</th>
<th>CULTURE POSITIVE</th>
<th>ORGANISMS SEEN ON HISTOLOGY</th>
<th>CULTURE POSITIVE AND / OR ORGANISMS SEEN ON HISTOLOGY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenal ulceration</td>
<td>67</td>
<td>54 (81%)</td>
<td>63 (94%)</td>
<td>65 (97%)*</td>
</tr>
<tr>
<td>Duodenal erosions</td>
<td>20</td>
<td>17 (85%)</td>
<td>19 (95%)</td>
<td>19 (95%)</td>
</tr>
<tr>
<td>Duodenal erythema</td>
<td>10</td>
<td>7 (70%)</td>
<td>9 (90%)</td>
<td>9 (90%)</td>
</tr>
<tr>
<td>Gastric ulceration</td>
<td>21</td>
<td>10 (48%)</td>
<td>13 (62%)</td>
<td>13 (62%)</td>
</tr>
<tr>
<td>Gastric erosions</td>
<td>3</td>
<td>1 (33%)</td>
<td>1 (33%)</td>
<td>1 (33%)</td>
</tr>
<tr>
<td>Gastric erythema</td>
<td>13</td>
<td>6 (46%)</td>
<td>8 (62%)</td>
<td>8 (62%)</td>
</tr>
<tr>
<td>Other (including oesophagitis, hiatus hernia)</td>
<td>20</td>
<td>14</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Normal (no abnormality detected)</td>
<td>70</td>
<td>37 (53%)</td>
<td>51 (73%)</td>
<td>53 (76%)**</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>224</td>
<td>146 (65%)</td>
<td>179 (80%)</td>
<td>183 (82%)</td>
</tr>
</tbody>
</table>

* 2 culture positive only
** 2 culture positive only
<table>
<thead>
<tr>
<th>HISTOLOGICAL DIAGNOSIS</th>
<th>TOTAL</th>
<th>CULTURE POSITIVE</th>
<th>ORGANISMS SEEN ON HISTOLOGY</th>
<th>CULTURE POSITIVE AND / OR ORGANISMS SEEN ON HISTOLOGY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic superficial gastritis with activity</td>
<td>177</td>
<td>134 (75%)</td>
<td>166 (94%)</td>
<td>167 (94%)</td>
</tr>
<tr>
<td>Chronic superficial gastritis with no activity</td>
<td>12</td>
<td>8 (67%)</td>
<td>9 (75%)</td>
<td>10 (83%)</td>
</tr>
<tr>
<td>Chronic superficial gastritis with intestinal metaplasia</td>
<td>5</td>
<td>1 (20%)</td>
<td>3 (60%)</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>TOTAL NUMBER OF PATIENTS WITH GASTRITIS</td>
<td>194</td>
<td>143 (74%)</td>
<td>178 (92%)</td>
<td>180 (93%)</td>
</tr>
<tr>
<td>Normal (no abnormality detected)</td>
<td>30</td>
<td>3 (10%)</td>
<td>1 (3%)</td>
<td>3 (10%)</td>
</tr>
</tbody>
</table>
**TABLE III**  THE ASSOCIATION BETWEEN CAMPYLOBACTER PYLORI AND SEX OF PATIENTS (FIRST STUDY)

<table>
<thead>
<tr>
<th></th>
<th>CULTURE POSITIVE AND / OR ORGANISMS SEEN ON HISTOLOGY</th>
<th>CULTURE NEGATIVE AND ORGANISMS NOT SEEN ON HISTOLOGY</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>130 (86%)</td>
<td>22 (14%)</td>
<td>152</td>
</tr>
<tr>
<td>Female</td>
<td>53 (74%)</td>
<td>19 (26%)</td>
<td>72</td>
</tr>
<tr>
<td>TOTAL</td>
<td>183</td>
<td>41</td>
<td>224</td>
</tr>
</tbody>
</table>

**TABLE IV**  THE ASSOCIATION BETWEEN CAMPYLOBACTER PYLORI AND RACE OF PATIENTS (FIRST STUDY)

<table>
<thead>
<tr>
<th></th>
<th>CULTURE POSITIVE AND / OR ORGANISMS SEEN ON HISTOLOGY</th>
<th>CULTURE NEGATIVE AND ORGANISMS NOT SEEN ON HISTOLOGY</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indian</td>
<td>110 (84%)</td>
<td>21 (16%)</td>
<td>131</td>
</tr>
<tr>
<td>African</td>
<td>73 (78%)</td>
<td>20 (22%)</td>
<td>93</td>
</tr>
<tr>
<td>TOTAL</td>
<td>183</td>
<td>41</td>
<td>224</td>
</tr>
<tr>
<td>METHOD</td>
<td>C. PYLORI POSITIVE <em>(n=84)</em></td>
<td>C. PYLORI NEGATIVE *<em>(n=16)</em></td>
<td>SENSITIVITY</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-----------------------------</td>
<td>-------------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td></td>
<td>No. POSITIVE</td>
<td>No. NEGATIVE</td>
<td>No. POSITIVE</td>
</tr>
<tr>
<td>STOLOGY</td>
<td>82</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>LTURE</td>
<td>57</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>STRIC UREASE ASSAY (37°C)</td>
<td>60</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>STRIC UREASE ASSAY (RT°C)</td>
<td>46</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>TI-C. PYLORI-IgG</td>
<td>69</td>
<td>15</td>
<td>10</td>
</tr>
</tbody>
</table>

* C. pylori positive = organism either cultured and/or seen on histology

** C. pylori negative = organism neither cultured nor seen on histology
### TABLE VI: COMPARISON OF CHRONIC, SUPERFICIAL ACTIVE GASTRITIS (CSAG) WITH THE VARIOUS METHODS TO DETECT THE PRESENCE OF C. PYLORI IN 100 PATIENTS (SECOND STUDY)

<table>
<thead>
<tr>
<th>METHOD</th>
<th>CSAG (n = 90)</th>
<th>NORMAL HISTOLOGY (n = 10)</th>
<th>SENSITIVITY</th>
<th>SPECIFICITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. POSITIVE</td>
<td>No. NEGATIVE</td>
<td>No. POSITIVE</td>
<td>No. NEGATIVE</td>
</tr>
<tr>
<td>ISTOLOGY</td>
<td>80</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>ULC TURE</td>
<td>55</td>
<td>35</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>ASTRIC UREASE ASSAY (37°C)</td>
<td>58</td>
<td>32</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>ASTRIC UREASE ASSAY (RT°C)</td>
<td>46</td>
<td>44</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>NTI-C. PYLORI-IgG</td>
<td>68</td>
<td>22</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>METHOD</td>
<td>C. PYLORI POSITIVE * ( (n = 88) )</td>
<td>C. PYLORI NEGATIVE ** ( (n = 12) )</td>
<td>SENSITIVITY</td>
<td>SPECIFICITY</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------------------</td>
<td>---------------------------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td></td>
<td>No. POSITIVE</td>
<td>No. NEGATIVE</td>
<td>No. POSITIVE</td>
<td>No. NEGATIVE</td>
</tr>
<tr>
<td>ISTOLOGY</td>
<td>85</td>
<td>3</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>ULTURE</td>
<td>70</td>
<td>18</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>ASTRIC UREASE ASSAY ( (37^\circ C) )</td>
<td>62</td>
<td>26</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>ONE-MINUTE&quot; UREASE ASSAY</td>
<td>76</td>
<td>12</td>
<td>0</td>
<td>12</td>
</tr>
</tbody>
</table>

* C. pylori positive = organism either cultured and / or seen on histology
** C. pylori negative = organism neither cultured nor seen on histology
PLATE 1: HAEMATOXYLIN AND EOSIN- (H & E) STAINED SECTION OF GASTRIC MUCOSA (1000X), SHOWING _C. PYLORI_ IN THE PRESENCE OF CHRONIC ACTIVE GASTRITIS

PLATE 2: WARTHIN-STARRY STAINED SECTION OF GASTRIC MUCOSA (1000X), SHOWING _C. PYLORI_ ADJACENT TO THE EPITHELIUM IN A GASTRIC GLAND.
PLATE 3: TINY, COLOURLESS COLONIES OF *C. PYLORI* ON CHOCOLATE AGAR; POSITIVE OXIDASE AND UREASE REACTIONS ARE SHOWN
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